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BIOACCUMULATION OF DDT IN A MARINE POLYCHAETE, THE CONVEYOR-BELT DEPOSIT FEEDER *HETEROMASTUS FILIFORMIS* (CLAPAREDE)

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Abstract

To better understand the fate of toxic pollutants within the sediment column and their uptake by the benthic community, the effects of several sub-lethal DDT concentrations were determined on *Heteromastus filiformis* (a marine head-down deposit feeder) under laboratory conditions. Net DDT uptake by this polychaete (measured at 5, 11, and 28 days) increased through time for all treatments (2, 4, and 8 $\mu g\ g^{-1}$ DDT in sediments) and reached concentrations of 240, 500, and 870 μg of DDT per g of lipid, respectively, at the end of the experiment. Biota sediment accumulation factor (BSAF) for DDT (organism concentration normalized to lipid content divided by sediment concentration normalized to the sediment total organic carbon content) ranged from 0.4 to 0.8. Sediment reworking rate of *H. filiformis* measured by fecal pellet production was reduced only when the worms were exposed to the highest concentration (8 $\mu g\ g^{-1}$ DDT after 28 d exposure). Initially, fecal pellets contained 5 to 8 times greater DDT concentrations than the spiked sediments, but these values decreased at 11 and 28 days. In this study, DDT reduced the feeding rate of *H. filiformis* at a relatively low DDT concentration (8 $\mu g\ g^{-1}$), compared to concentrations reported for marine sediments, after a relatively short time (28 days), and the buried DDT was transported to the sediment-water interface through the fecal pellets.

Introduction

Ecological studies of marine deposit feeders, particularly conveyor-belt deposit feeders, have suggested that these organisms play a major role in removal and burial of particulate and dissolved organic matter during early diagenetic processes (Rhoads 1974, Aller 1980, Berner 1980, Levinton 1989). These organisms can feed at 10's of centimeters within the sediment column and deposit the ingested particles at the sediment-water interface (Rhoads 1974). Their important ecological role in marine and freshwater food chains has been a major reason for studying their behavior, including responses to ecological stressors such as anthropogenic chemicals

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introduced by rivers and coastal areas to lakes and open oceans (Dexter and Pavlou 1973, Hom *et al.* 1974, Elder *et al.* 1976).

Many organic pollutants (e.g., DDT, Endrin, Aldrin) are strongly bound to sediments, particularly to the organic matter fraction and are highly refractive, resulting in a long sediment residence time (years) after deposition (Elder *et al.* 1979). Thus, contaminants associated with sediments are potentially available to benthic organisms even after these compounds are no longer produced or introduced into the aquatic environment. For instance, DDT and PCB concentrations continue to be found in bottom sediments and bottom feeding fish from coastal areas at $\mu g\ g^{-1}$ concentrations (Verschueren 1983). Values from 10 to 150 x 10^3 $\mu g\ g^{-1}$ DDT for bottom sediments have been reported in Southern California despite a marked decrease in their input (Young *et al.* 1977, 1991).

Several studies have demonstrated that organic contaminant bioaccumulation by freshwater deposit feeders is several times higher than the concentration of the compound in the sediments (Fowler *et al.* 1978, Appleby and Brinkhurst 1970, Bailey and Liu 1980, Oliver 1984, Karickhoff and Morris 1985, Klump *et al.* 1987). However, marine deposit feeders have not been extensively studied, and only a few investigations have been carried out on marine infaunal polychaetes. For instance, the marine polychaete *Nereis diversicolor* was shown to incorporate PCB's mainly from contaminated sediments to greater than 3 times the sediment concentration (Elder *et al.* 1979) rather than accumulating PCB's from the contaminated overlying seawater (Fowler *et al.* 1978). This observation is interesting because most members of the family Nereididae are omnivorous and they burrow and crawl in the sediment in search of food (Goerke 1966, Fauchald and Jumars 1979). Therefore, they do not necessarily obtain their food by ingesting sediments. Thus, true deposit feeders, such as head-down (conveyor-belt) deposit feeders (e.g. *Heteromastus filiformis*), that directly ingest sediment are expected to have even higher bioaccumulation.

In this study, *Heteromastus filiformis* was exposed to sediment-associated DDT at several sub-lethal concentrations to examine the potential effects on the sediment reworking rate (fecal pellet production) and to measure the DDT bioaccumulation.

Materials and Methods

Sediment and polychaete collection

Marine sediments and polychaetes were collected with a brass core tube (50 cm long and 25 cm diameter) from a subtidal channel in Barnstable Harbor (Cape Cod, Massachusetts). The sediment samples were placed in plastic bags in a cooler. The animal cores were carefully sorted by hand to remove and collect only whole individuals of *Heteromastus filiformis*. The polychaetes were kept in glass vials containing seawater and a few mg of wet sediments and then placed into a seawater filled cooler. The collected sediments and animals were transported to the laboratory within 24 h. Once in the laboratory, the animals were maintained at 14 °C, the temperature of collection, and constantly aerated. The sediments were kept in the dark at 4 °C.

Sediment preparation

The sediments (composed of >60% mud and fine sand) were sieved through a 250 μm mesh to remove macroinfauna. The particle size of the sediment is within that ingested by *Heteromastus filiformis* (Cadée 1979). The sediment (free of macroinfauna) was split into four aliquots of about 1.5 L each. Three DDT concentrations were tested in this experiment: mixtures of non-radio labeled and ^{14}C-DDT, at 1 μCi of labeled DDT per treatment concentration, were dissolved in 2 mL of acetone to provide final sediment concentrations of approximately 2, 4 and 8 $\mu g\ g^{-1}$. The solutions were then added to the sediments under constant suspension and stirred at room temperature for 2 h. The spiked sediments were allowed to settle at 4 °C overnight. To remove the acetone, the overlying water was withdrawn and replaced with clean seawater. The rinsed sediment slurries were then stirred for an additional 2 h and allowed to settle overnight. The overlying water was again discarded. The fourth aliquot of sediment, used as the control treatment, was subjected to the same procedure, but only 2 mL of acetone with no DDT was added. Finally, DDT concentrations were measured for each treatment by extracting the pollutant from ~100 mg of sediment (wet weight basis) using 12 mL of scintillation cocktail (Research Products International 3a70B). The mixture of sediment and scintillation cocktail was sonicated every other second for 3 minutes. The extracted DDT in the scintillation cocktail was filtered, and the filtered cocktail was counted for ^{14}C activity on an LKB 1217 scintillation counter. The samples were corrected for quench using the external standards ratio method after subtracting background. The amount of radioactivity was used to calculate DDT concentrations on a dry weight sediment basis based on the specific activity after the isotopic dilution and the wet to dry weight ratio for the sediment. The initial measured DDT concentrations in the experimental sediments were: 2.87 ± 0.06 $\mu g\ g^{-1}$ (treatment I), 4.5 ± 0.5 $\mu g\ g^{-1}$ (treatment II) and 8.5 ±1.2 $\mu g\ g^{-1}$ (treatment III; n = 3). All the treated sediments (DDT spiked and control) were kept at 4 °C (for circa 3 weeks) until the start of the experiment.

Experimental design

Twenty-four beakers (200 mL) were each filled with approximately 100 mL of wet sediment (six beakers per treatment: control, treatments I, II and III). Three additional beakers containing spiked sediments were used as controls (no animals) to determine DDT degradation over the course of the experiment. All the experimental beakers were placed in aquaria at 14°C, and the aquaria water was aerated to prevent suspending the sediment. After 24 h, three *Heteromastus filiformis* were placed in each of the 24 treatment beakers. To study DDT uptake, two beakers per treatment were withdrawn after 5, 11, and 28 days. Each beaker was sampled for sediment (to measure DDT concentration and degradation and total organic carbon), fecal material (to determine the defecation rate and fecal DDT concentration), and animals (for DDT concentration and lipid content).

Sediment DDT concentration was determined following the same method as described earlier. To measure DDT degradation, sediment samples (200 mg) were first extracted with scintillation cocktail (50 mL), centrifuged, and the cocktail decanted. The sediments were subsequently extracted with cyclohexane, and the solvent was decanted. The liquid phases were combined and reduced to about 5 mL by rotary flash evaporation. The remaining solvent was then transferred to a centrifuge tube and further reduced to approximately 2 mL under a stream of nitrogen. The concentrated extract was streaked onto a silica gel TLC plate and developed with benzene and acetone (4:1). After development, the plate was viewed under UV light, and the bands of interest (DDT, DDE, and DDD) were marked for comparison with standard DDT, DDE, and DDD. The silica gel was then sectioned every 1 cm, scraped from the plate, and placed in a vial with scintillation cocktail. The radioactivity was measured to obtain a quantitative estimation of the DDT degradation. Total organic carbon (TOC) in sediment from each experimental beaker was determined by high temperature oxidation of 10-15 mg of dry sediment, free of carbonates (previously removed with 1N HCl; 10% volume), on a Perkin-Elmer model 2400 CHN analyzer.

The defecation rate was estimated at various time periods as the quantity of fecal pellets produced per individual of *H. filiformis* per unit time (g pellets $worm^{-1}$ h^{-1}). The pellets were carefully removed from the sediment surface with a Pasteur pipette and placed in glass vials. DDT was first extracted from the pellets with scintillation cocktail and homogenized by sonication following the same procedure as described for sediments. The sonicated sample was then filtered through a pre-weighed filter (FG/C Gelman glass filter). Fecal pellet production was determined by subtracting the filter's weight from the collected solid fraction (dry weight after 24 h at 104 °C). The filtered scintillation cocktail was analyzed for ^{14}C activity to determine the DDT concentration.

Each polychaete was carefully collected and placed into clean seawater for 2 hours to empty gut contents based

on visual inspection, dried for 24 h (at 38 °C), and weighed. Lipid content and DDT concentration were determined on each dried animal. The lipid fraction was isolated following the micro-gravitational technique developed by Gardner *et al.* (1985). The DDT content of this isolate was analyzed by liquid scintillation counting (LSC). The remaining body tissues, after lipids were extracted, were analyzed for DDT by placing the tissues in scintillation cocktail, sonicating, and LSC. The sum of both extractable and non-extractable DDT was used as total DDT concentration in the animal.

Measured DDT concentrations were expressed on a μg g^{-1} dry weight basis for all samples. The biota-sediment accumulation factor (BSAF) of DDT for *H. filiformis* was defined as the lipid normalized DDT concentration in the organism divided by the organic carbon normalized concentration of DDT in the sediment.

Kinetics

Accumulation and loss kinetics were estimated from the accumulation data by fitting the data to a first order uptake and loss model (Equation 1). The concentration in the sediment was essentially constant so that a concentration-based model could be used,

$$C_a = \frac{k_s C_s}{k_e}(1 - e^{-k_e t}) \quad (1)$$

where C_a is the concentration of DDT in the animal (ng g^{-1} dry weight organism), k_s is the uptake clearance (g dry sediment g^{-1} dry wt organism h^{-1}), C_s is the DDT concentration in the sediment (ng g^{-1} dry weight), k_e is the elimination rate constant (h^{-1}), and t is time (h). The data were fitted to the above equation using non-linear least square fit from SYSTAT® (Wilkinson 1990).

Results

Sediments

The sediment TOC was less than 1% (Table 1). These values are within the TOC range for sediments 10-15 cm below the sediment-water interface, where *H. filiformis* typically feeds (Clough and Lopez 1993). Percent TOC decreased in all the treatments over the course of the experiment (20-50% TOC decrease after 28 days; Table 1). DDT concentrations in sediments did not change during the same period and no degradation was observed. The radiopurity of DDT was approximately 98% after 28 days.

Defecation rate

Survival was 100% at all sampling times (5, 11, and 28 days). Defecation rates ranged from 0.2 to 1.3 mg animal^{-1} h^{-1}. For each treatment (control, I, II, and III; Figure 1), defecation rates increased throughout the experiment; this increase was more pronounced between 5 and 11 days. At the end of the experiment, polychaetes from treatment III had a significantly lower defecation rate than those from treatments I, II, and control (ANOVA, $p < 0.05$). At 5 and 11 days, no significant differences were observed between treatments or sampling points.

Table 1. Experimental sediment characteristics: Initial (0d) and final (28d) TOC (% of sediment and average DDT concentrations (μg g^{-1}) throughout the experiment.

Treatment	Total Organic Carbon (%)[1]		Sediment DDT[2]
	Initial	Final	μg g^{-1}
Control	0.58 ± 0.1	0.43 ± 0.03	-
Treatment I	0.96 ± 0.1	0.50 ± 0.24	2.5 ± 0.3
Treatment II	0.75 ± 0.3	0.44 ± 0.08	4.0 ± 0.4
treatment III	0.79 ± 0.1	0.54 ± 0.27	7.5 ± 0.9

1. Mean ± SD, n=4

2. Mean ± SD, n=9

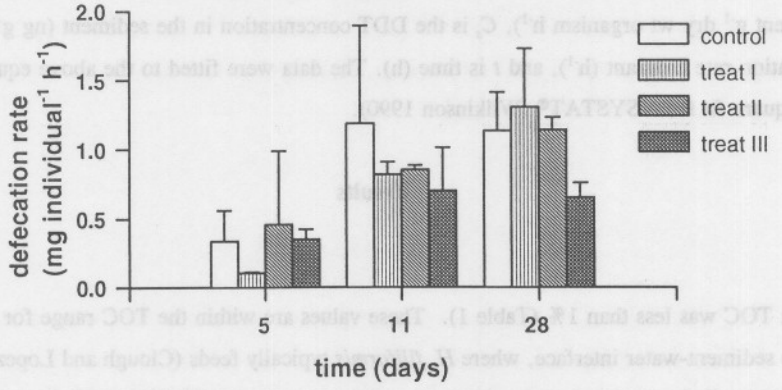

Figure 1. Defecation rate of *Heteromastus filiformis*. Plotted are mean values ± range interval (n = 2).

DDT Uptake

The lipid content of *H. filiformis* ranged from 0.6 to 6.7% of total dry body weight with a mean value of 3.4 ± 0.6 % (n = 25). Of the total DDT measured in the organisms, 88% (± 1%) was extracted with the lipids. The amounts of DDT detected in *H. filiformis* increased throughout the experiment for all the treatments, reaching maximum values after 28 days (240 ± 67, 500 ± 72 and 870 ± 229 μg DDT g^{-1} lipid for treatments I, II and III respectively). The differences observed between treatments were not significant after 5 days (Kruskal-Wallis; $p > 0.05$); at 11 days polychaetes from treatment III had significantly higher amounts of DDT in their bodies than animals from treatments I and II, while all the treatments were significantly different at the end of the experiment (ANOVA; $p < 0.05$; Figure 2).

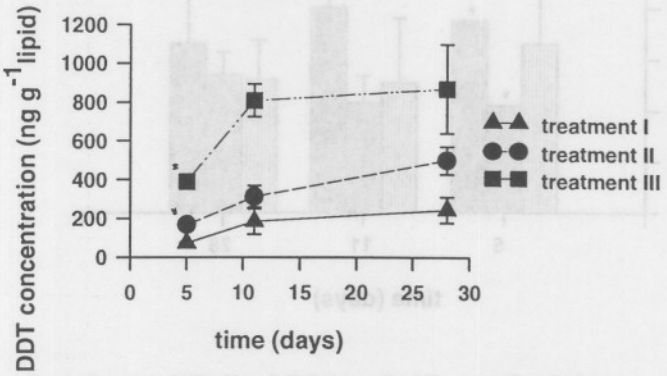

Figure 2. DDT uptake by *H. filiformis* expressed as the amount of DDT normalized to the fraction of lipid. Plotted are mean values ± 1 standard deviation (n = 6). (*) = one observation only.

Biota-sediment accumulation factors (BSAF, ratio of lipid normalized DDT concentration in worms divided by carbon normalized DDT concentration in sediment) ranged from 0.4 to 0.8. They were more or less constant throughout the experiment for each treatment (Figure 3); no significant differences were observed among treatments and sampling days (ANOVA; $p > 0.05$).

The sediment uptake clearance of DDT by *H. filiformis* was essentially constant across the range of DDT concentrations (Table 2). These values (at 14 °C) are similar to those observed for *Diporeia* spp. exposed to DDT contaminated sediments at 10 °C with similar levels of organic carbon (Harkey *et al.* 1994). The elimination rate constants indicate that steady state should have been achieved in 6 to 10 days. This accounts for the essentially constant BASF values observed at our chosen sampling times.

Fecal Pellets

Fecal pellets produced by *H. filiformis* always contained more DDT (3-8 times) than was present in the experimental sediment (Table 1 & Figure 4). A gradual decrease in the amount of DDT was observed in the course of the experiment reaching values of 11.5, 13.4, and 31.3 μg g-1 for treatments I, II, and III respectively at 28 days.

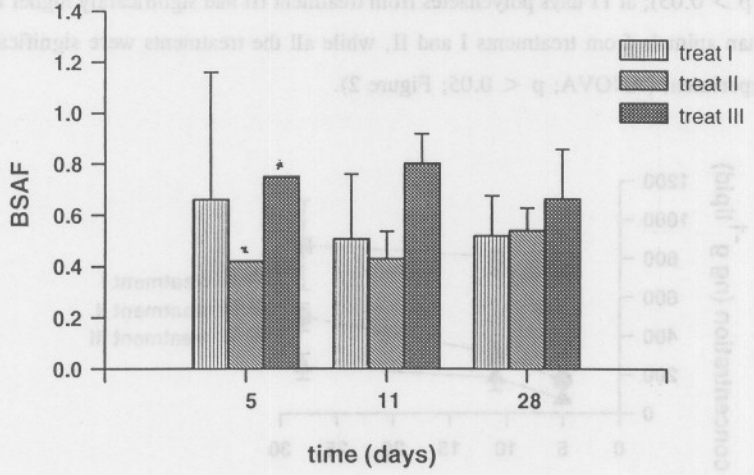

Figure 3. Biota-sediment accumulation factors (BSAF) of DDT for *H. filiformis*. Plotted are mean values ± 1 standard deviation (n = 6). (*) = one observation only.

Discussion

The uptake of DDT by *H. filiformis* increased throughout the experiment (Figure 2), and the total accumulation was directly related to the concentration of DDT in the sediments (Table 1). After 5 days, the polychaetes contained 40 times more DDT in their body than was present in the sediment, regardless of the initial quantity of DDT in the microcosms; this proportion increased to circa 115 times at the end of the experiment for all three treatments. The animals incorporated the pollutant at similar rates independent of the amount of DDT in the sediment as shown by the nearly constant uptake clearance and elimination coefficients (Table 2). This was also reflected by the bioaccumulation factors calculated for this species (Figure 3). Concentration factors of DDT by *H. filiformis,* expressed on a wet weight basis, ranged from 13 to 57; these values were considerably higher than those calculated for PCBs in other polychaetes (3 - 10 for *Nereis virens*; Courtney and Langston 1978 and 3.5 for *N. diversicolor*; Fowler *et al.* 1978). These differences probably reflect the fact that these

two genera belong to different feeding guilds; *Nereis* is an omnivorous and a facultative filter feeder, while *Heteromastus filiformis* is a true deposit feeder. Thus, nereids, unlike *Heteromastus*, do not depend entirely on sediment ingestion for food. The fate of a sediment-bound pollutant, such as DDT, seems to depend on the feeding guild to which macroinfauna belong. DDT bioaccumulated by true deposit feeders might reach the next trophic level of the marine food chain with concentrations several orders of magnitude higher than in the sediments.

Table 2. Uptake clearance and elimination coefficients for *H. filiformis* exposed to DDT contaminated sediments.

Kinetic Coefficients	Treatment I	Treatment II	Treatment III
Uptake Clearance (k_s)	0.013 ± 0.006[1]	0.014 ± 0.003	0.024 ± 0.008
Elimination Constant (k_d)	0.004 ± 0.003[1]	0.003 ± 0.001	0.006 ± 0.003

1. Standard error of regression

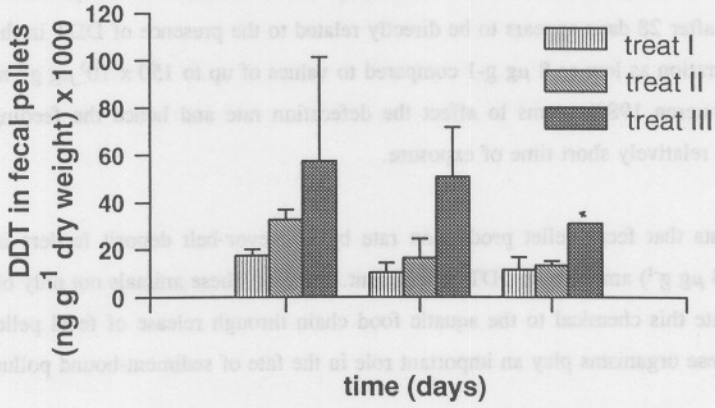

Figure 4. DDT concentrations measured in fecal pellets of *H. filiformis*. Plotted are mean values ± range interval (n = 2). (*) = one observation only.

The observed increase in defecation rate of *Heteromastus filiformis* after 5 days in all the treatments and control (Figure 1) is probably a response to a decrease in the organic matter content (20-50% TOC decrease during

the experiment; Table 1) rather than an effect of DDT in the sediments. Marine deposit feeders are known to regulate their ingestion rate in response to changes in the organic content of their food supply, to maintain an optimal intake of nutrients (Taghon 1981). As the organic matter concentration declined, *H. filiformis* ingested more sediments to sustain its metabolism and, therefore, produced more fecal pellets. This increasing defecation rate corresponds with the decrease of DDT concentration in the fecal pellets during the experiment (Figure 4). This correspondence might be due to differences in DDT binding in the ingested particles or a difference in assimilation efficiency from the later ingested particles. The reduced concentration of DDT in the fecal pellets occurs even though the concentration of DDT in the whole sediment does not change. Probably with a larger pool of labile organic matter available for the organisms, the defecation rate of *H. filiformis* would have remained constant throughout the experiment with a correspondingly high DDT concentration in the feces. When DDT is egested in the feces at the sediment water interface, it can also enter the aquatic food chain directly through coprophagy by surface deposit feeders as the compound would be readily available in a concentrated form to micro-, meio-, and macroorganisms. This ecological mechanism is not trivial when one considers that *H. filiformis* may be found in densities of $> 5000\ m^{-2}$ (Shaffer 1983).

Given that the experimental conditions were identical for all the treatments and that no death occurred during the experiment, the lower pellet production rate of *H. filiformis* in treatment III compared to those of treatments I, II, and control after 28 days appears to be directly related to the presence of DDT in the sediment. Thus, DDT at a concentration as low as 8 μg g-1 compared to values of up to $150 \times 10^3\ \mu g\ g^{-1}$ measured in marine sediments (Verschueren 1983) seems to affect the defecation rate and hence the feeding behavior of this polychaete after a relatively short time of exposure.

This study suggests that fecal pellet production rate by conveyor-belt deposit feeders is decreased in the presence of low ($8\ \mu g\ g^{-1}$) amounts of DDT in sediment. Further, these animals not only bioaccumulate DDT but also incorporate this chemical to the aquatic food chain through release of fecal pellets at the sediment surface. Thus, these organisms play an important role in the fate of sediment-bound pollutants in the marine environment.

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